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PB-277 183

Kepone (Trademark) Bioconcentration, Accumulation, Loss, and Transfer through Estuarine Food Chains

Environmental Research Lab, Gulf Breeze, Fla

1977

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO.	2.	3. PB 277 183
EPA-600/J-77-074	JOURNAL ARTICLE	

4. TITLE AND SUBTITLE	5. REPORT DATE
KEPONE ACCUMULATION, TRANSFER, AND LOSS THROUGH ESTUARINE FOOD CHAINS	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S)	8. PERFORMING ORGANIZATION REPORT NO.
Bahner, Lowell H., Alfred J. Wilson, James M. Sheppard, James M. Patrick, Larry R. Goodman, and Gerald E. Walsh	
9. PERFORMING ORGANIZATION NAME AND ADDRESS	10. PROGRAM ELEMENT NO.
Same as box 12.	LEA615
	11. CONTRACT/GRANT NO.
12. SPONSORING AGENCY NAME AND ADDRESS	13. TYPE OF REPORT AND PERIOD COVERED
ENVIRONMENTAL RESEARCH LABORATORY, GULF BREEZE OFFICE OF RESEARCH AND DEVELOPMENT U.S. ENVIRONMENTAL PROTECTION AGENCY GULF BREEZE, FL 32561	final
	14. SPONSORING AGENCY CODE EPA/600/04

15. SUPPLEMENTARY NOTES

REFERENCE: CHEASAPEAKE SCIENCE VOL. 18, NO. 3, pp. 299-308, September 1977

16. ABSTRACT

Accumulation, transfer, and loss of Kepone in estuarine organisms were studied in laboratory bioassays. Kepone was bioconcentrated by oysters (Crassostrea virginica), mysids (Mysidopsis bahia), grass shrimp (Palaemonetes pugio), sheepshead minnows (Cyprinodon variegatus), and spot (Leiostomus xanthurus), from concentrations as low as 0.023 µg/l seawater. Bioconcentration factors ranged from 10 to 340 in static exposures and 900 to 13,500 in flow-through bioassays, and were dependent on species and exposure duration. Depuration of Kepone from oysters in Kepone-free water was rapid (35% loss in 24 hours); however, depuration of Kepone was slow in crustaceans and fish, with tissue concentrations decreasing 30-50% in 24-28 days. Bioaccumulation factors (concentration of Kepone in predator/concentration in prey) at 30 days were equal (0.85 spot/mysid; 0.53 mysid/brine shrimp) in the high and low concentrations tested. The initial bioconcentration of Kepone from water by plankton was the dominant source of Kepone to each member of this food chain, but significant (>85%) quantities of Kepone transferred from prey to predatory fish.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Bioassay Pesticides Kepone Shrimp Fish		

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U. S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS *(This Report)*	21. NO. OF PAGES
release to public	unclassified	10
	20. SECURITY CLASS *(This page)*	22. PRICE
	unclassified	A02-A01

EPA Form 2220-1 (9-73)

EPA-600/J-77-074
JOURNAL ARTICLE

Chesapeake Science Vol. 18, No. 3, p. 299-308 September 1977

Kepone® Bioconcentration, Accumulation, Loss, and Transfer through Estuarine Food Chains.[1]

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ABSTRACT: Accumulation, transfer, and loss of Kepone in estuarine organisms were studied in laboratory bioassays. Kepone was bioconcentrated by oysters (*Crassostrea virginica*), mysids (*Mysidopsis bahia*), grass shrimp (*Palaemonetes pugio*), sheepshead minnows (*Cyprinodon variegatus*), and spot (*Leiostomus xanthurus*), from concentrations as low as 0.023 μg/l seawater. Bioconcentration factors ranged from 10 to 340 in static exposures and 900 to 13,500 in flow-through bioassays, and were dependent on species and exposure duration.

Depuration of Kepone from oysters in Kepone-free water was rapid (35% loss in 24 hours); however, depuration of Kepone was slow in crustaceans and fish, with tissue concentrations decreasing 30–50% in 24–28 days.

Oysters, fed *Chlorococcum* containing approximately 34 μg Kepone/g wet weight, attained 0.21 μg Kepone/g (wet tissue) in 14 days, but when fed Kepone-free plankton, depurated Kepone to below detectable concentrations (<.02 μg/g) within 10 days.

Spot obtained Kepone when fed live mysids that had grazed on Kepone-laden brine shrimp. Kepone residues (1.05 μg/g wet tissue) in these fish approached the concentration of their food (1.23 μg/g wet tissue); at the lower concentration tested, Kepone concentrations below detection limits (<.2 μg/g) in prey accumulated in the predator to detectable concentrations (0.02 μg/g) within 30 days. Bioaccumulation factors (concentration of Kepone in predator/concentration in prey) at 30 days were equal (0.85 spot/mysid; 0.53 mysid/brine shrimp) in the high and low concentrations tested. The initial bioconcentration of Kepone from water by plankton was the dominant source of Kepone to each member of this food chain, but significant (>85%) quantities of Kepone transferred from prey to predatory fish.

Introduction

Contamination of the James River water, sediments, and biota with Kepone has prompted research to help define the routes of transfer of the insecticide from water through selected estuarine trophic levels. Biota of the James River estuary and Chesapeake Bay contain Kepone (Hansen et al. 1976), apparently due to transport of the chemical downstream from the freshwater portion of the river. Since no convenient method existed to assess the rate of Kepone movement in the biota of the James River and Chesapeake Bay, laboratory bioconcentration from water and bioaccumulation from food experiments were designed to determine the rates and magnitudes of Kepone accumulated from water and food by selected estuarine organisms. It is important to determine the accumulation of Kepone from water and food by various estuarine species, so that the information can be used in the decision-making processes that may affect the water quality for the biota in the Chesapeake Bay region, or limit transfer of Kepone to seafoods consumed by man. The alga, oyster, mysid, shrimp, and fish used in

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[1] Contribution No. 294, Environmental Research Laboratory, Gulf Breeze, FL.

our experiments are representative of many ecologically important species. The top consumers in our food chains tested are endemic to both the Chesapeake Bay area and northern Gulf of Mexico and are commercially important human food items. *Leiostomus xanthurus*, the top carnivorous species of one laboratory food-chain, provides an estimated 21.5 million pounds annual recreational catch for fishermen of the Middle Atlantic states (U.S. Department of Commerce 1975).

This study provides information about: (1) the rates and magnitudes of Kepone accumulation from water by estuarine biota; (2) rates of Kepone depuration by animals in Kepone-free water; and (3) rates of Kepone transfer through laboratory food chains.

Methods

Bioconcentration of Kepone from Water by Estuarine Organisms

Oysters

Eastern oysters (*Crassostrea virginica*) were exposed to Kepone (88% pure) in a 56-day, flow-through bioassay to determine the rates of uptake and depuration of this insecticide. Seawater (mean temperature 14.2 C, range 9–20 C; mean salinity 15 ‰, range 4–22 ‰) was pumped from Santa Rosa Sound, Florida, into a constant-head trough in the laboratory. Approximately 440 l/hour was delivered by siphons to each of three 166 l aquaria. Oysters (100/aquarium) were not fed but could obtain plankton from the unfiltered seawater in which they were held. Stock solutions of Kepone in triethylene glycol (TEG) were metered into the two experimental aquaria at the rate of 10 ml/day. Measured concentrations of Kepone in the two experimental aquaria were 0.39 and 0.03 μg/l seawater. A control aquarium received 10 ml TEG/day.

Oysters (56 mm to 92 mm, umbo to distal valve edge height; $\bar{x}$ = 71.8 mm) were collected, acclimated to laboratory conditions for ten days, exposed to Kepone for 28 days, and then held for 28 days in Kepone-free seawater. Five oysters were sampled from each aquarium at 4 hours, 8 hours, 1 day, 8 days, and twice weekly thereafter to day 28. During the 28-day depuration portion of the test, oysters were sampled at similar intervals. Analysis methods for Kepone in water and tissues (whole-body, wet weight) were those of Schimmel and Wilson (1977).

Crustaceans

Mysids (*Mysidopsis bahia*), collected from laboratory cultures (Nimmo et al. 1977), were exposed to average measured concentrations of 0.026 or 0.41 μg Kepone/l seawater for 21 days (mean temperature 27.2 C, range 26–29 C; mean salinity 18 ‰, range 12–26 ‰) and in a second experiment, grass shrimp (*Palaemonetes pugio*), seined and acclimated to experimental conditions for 10 days, were exposed to average measured concentrations of 0.023 or 0.40 μg Kepone/l seawater in a 28-day, flow-through bioassay (mean temperature 27 C, range 26–29 C; mean salinity 25 ‰, range 21–28 ‰). Experimental methods used were those of Bahner et al. (1975). The grass shrimp were held for an additional 28-day period in clean seawater to assess depuration of the insecticide. Filtered seawater at a rate of approximately 60 l/hour flowed through each aquarium containing mysids or grass shrimp. Mysids and shrimp were fed 48-hour-old *Artemia* nauplii daily. Kepone content of mysids and shrimp was determined weekly during exposure and depuration. In a third experiment, grass shrimp were collected by seine from the Lafayette River estuary near Norfolk, Virginia, and were held in flowing seawater (mean temperature 25.5 C; mean salinity 15 ‰) in the laboratory to determine the extent of depuration of Kepone from field-exposed shrimp. These shrimp were analyzed for Kepone concentrations on days 7, 11, 17, and 21 after being transferred to flowing Kepone-free water in our laboratory.

Fishes

Sheepshead minnow (*Cyprinodon variegatus*) adults, acclimated to laboratory test conditions, were exposed to an average measured concentration of 0.05 μg Kepone/l of water (mean temperature 30 C;

mean salinity 15 ‰, range 8–26 ‰) for 28 days, using the methods of Hansen et al. (1977) and were held in Kepone-free water for an additional week. Ten fish, generally five females and five males, were sampled on days 0, 1, 3, 7, 14, 21, and 28 of exposure to Kepone and on day 7 of depuration.

Spot (*Leiostomus xanthurus*) were seined, acclimated for 10 days, exposed to average measured concentrations of 0.029 or 0.4 μg Kepone/l of filtered flowing seawater (mean temperature 23 C, range 21–24 C; mean salinity 18 ‰, range 9–24 ‰) for 30 days and allowed to depurate the chemical for 24 days. Composite samples of three fish were sampled each week for residue analysis. Spot, exposed to 0.4 μg Kepone/l seawater and allowed to depurate for 24 days, were dissected into liver, brain, gills, muscle, and offal (rest of body tissues) and analyzed for Kepone.

Fillets (including scaleless skin) and the remaining portions of sheepshead minnows and spot (wet weight) were analyzed for Kepone content. The data were summed to calculate concentrations in whole fish.

Bioaccumulation of Kepone in Food Chains Consisting of Estuarine Organisms

Algae-Oyster Food Chain

The green alga, *Chlorococcum* sp., contaminated with Kepone was used as food for oysters to determine if contaminated phytoplankton could be a significant source of the pesticide to oysters. *Chlorococcum* sp. was grown for 6 days in one liter of culture medium in 2800-ml Fernbach flasks according to the method of Hollister et al. (1975). Random cultures were dosed with 0.1 mg Kepone in acetone, while others served as controls after treatment with acetone alone. After 24 hours of exposure, algal cultures (mean wet weight = 0.15 g) were harvested by centrifugation and washed three times by resuspension in clean growth medium and centrifugation. The cells were resuspended in 5 l of seawater and fed to oysters by using the methods of Bahner and Nimmo (1976). Samples of the algae were analyzed daily for Kepone.

Rate of Kepone accumulation was determined by allowing oysters to feed on control or Kepone contaminated green algae in flowing seawater. Oysters for this study were collected and acclimated for 10 days to laboratory conditions in flowing seawater. Twenty-four oysters were placed in each of two aquaria (one control and one experimental) that received 60 l filtered seawater/hour (mean temperature 22 C, range 21–23 C; mean salinity 19 ‰, range 15–24 ‰) and were fed approximately 50 ml of the appropriate (control or contaminated) algal suspension at 15-minute intervals for 14 days. A 10-day depuration period followed the feeding study during which the oysters received raw, unfiltered, flowing seawater and no additional *Chlorococcum*. Oysters (n = 3 per sample) were analyzed for Kepone content on days 0, 7, 10, 14, 17, and 24 of the experiment.

Plankton-Mysid-Fish Food Chain

Transfer of Kepone from water to plankton to mysids to fish was investigated by feeding living brine shrimp nauplii that were contaminated with 2.33 μg Kepone/g tissue to mysids, that were then fed to spot, the top predator of this laboratory food chain. Juvenile spot were seined and acclimated according to the methods previously described for sheepshead minnows and spot. Mysids (*Mysidopsis bahia*), the intermediate food organism, were collected from laboratory cultures. Commercially available brine shrimp eggs were hatched during 48 hours in clean seawater or in seawater to which 0.005 or 0.1 mg Kepone/l was added (Bahner and Nimmo 1976). The brine shrimp were harvested daily and served as the "planktonic" food for the mysids. Approximately 40 mysids were distributed among each of six compartments of two 30-l aquaria. The compartments were separated by coarse nylon screen that allowed for flow of water and *Artemia* throughout each aquarium while confining the mysids to separate compartments. Mysids that had fed on *Artemia* for 72 hours were harvested from one compartment of each holding aquarium, rinsed with seawater, and fed to the spot. Each compartment was refilled with mysids to provide for subsequent feeding

periods. By this method, each of 12 juvenile spot, (average length 40 mm), in each of three 30-l glass aquaria, were fed 3 to 5 control or contaminated mysids daily. Aquaria containing spot received 60 l of seawater/hour to prevent anoxia and to minimize bioconcentration of Kepone depurated from the mysids. Water averaged 19 C (range 16–21) and 18 ‰ salinity (range 13–23 ‰). Brine shrimp, 30 to 45 mysids, 2 to 3 spot, and water from each aquarium were analyzed weekly for Kepone.

Results and Discussion

Bioconcentration from Seawater

Kepone was bioconcentrated from water by oysters, mysids, grass shrimp, sheepshead minnows, and spot in all concentrations tested (Figs. 1, 2, 3, 4, and 5; Table 1), and all species showed nearly equilibrated tissue concentrations of Kepone within 8 to 17 days after exposure to Kepone began in water. Bioconcentration factors for Kepone in these species ranged from 2,300 to 13,500 in long-term (>96 hrs) flow-through bioassays (Table 2). Kepone bioconcentrated in oysters to approximately 10,000 times the concentration in the exposure water within 19 days. Mysids bioconcentrated Kepone up to 13,000 times the amount measured in the exposure water. Each mysid (mean live-weight = 2.5 mg for 66 adults), exposed to 0.026 µg Kepone/l for 14 days contained approximately 5.9 ng Kepone; therefore, this amount of the chemical could enter food chains of estuarine predators that consumed each mysid. Stomachs of flounders from Chesapeake Bay (standard length 25 to 174 mm) con-

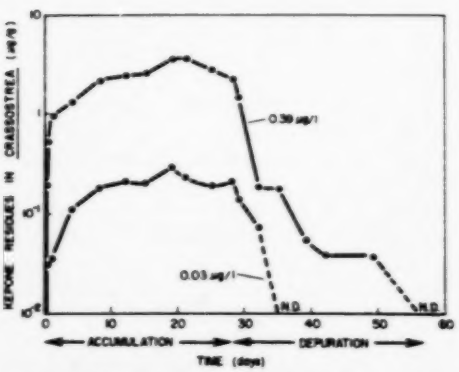

Fig. 1. Bioconcentration of Kepone from water containing average measured concentrations of 0.03 or 0.39 µg/l by oysters (*Crassostrea virginica*) exposed for 28 days, and its depuration by oysters placed in Kepone-free water for 28 days (mean temperature 14.2°C; mean salinity 15 ‰). ND = not detectable, <0.02 µg/g wet weight.

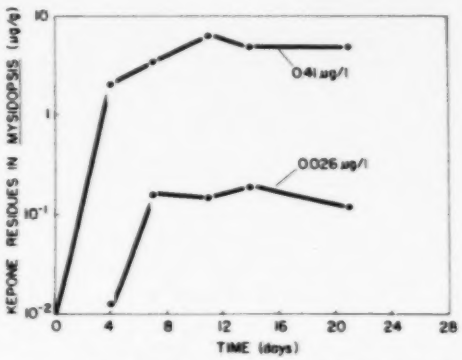

Fig. 2. Bioconcentration of Kepone from water containing average measured concentrations of 0.026 or 0.41 µg/l by mysids (*Mysidopsis bahia*) exposed for 21 days (mean temperature 27.2°C; mean salinity 18 ‰).

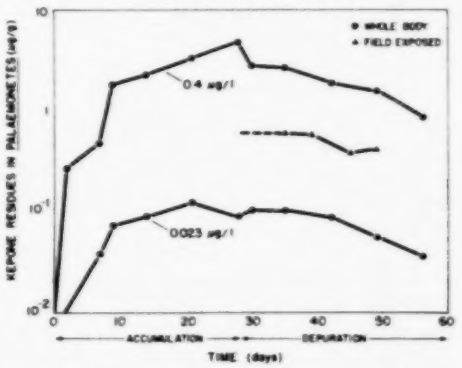

Fig. 3. Bioconcentration and depuration of Kepone in grass shrimp (*Palaemonetes pugio*) during 56-day study. Circles indicate concentrations of Kepone accumulated from water containing average measured concentrations of 0.023 or 0.4 µg/l by grass shrimp exposed in the laboratory for 28 days, and its depuration by shrimp placed in Kepone-free water for 28 days (mean temperature 27°C; mean salinity 25 ‰). Triangles indicate concentrations in shrimp collected from Lafayette River, Norfolk, Virginia and held in clean flowing seawater at the ERL, Gulf Breeze, for 21 days (mean temperature 25.5°C; mean salinity 15 ‰). Dashed line represents extrapolation to initial concentration at beginning of depuration.

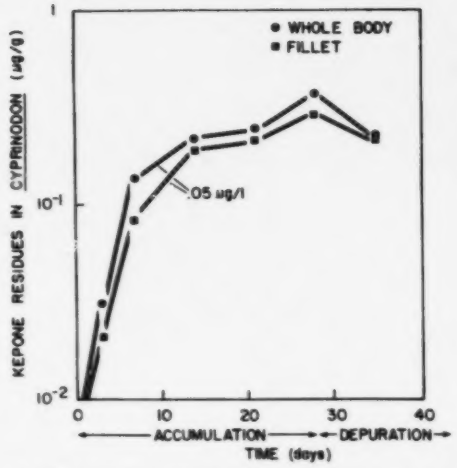

Fig. 4. Bioconcentration of Kepone from water containing average measured concentration of 0.05 µg/l by sheepshead minnows (*Cyprinodon variegatus*) exposed for 28 days, and its depuration from fish placed in Kepone-free water for 7 days (mean temperature 30°C; mean salinity 15 ‰).

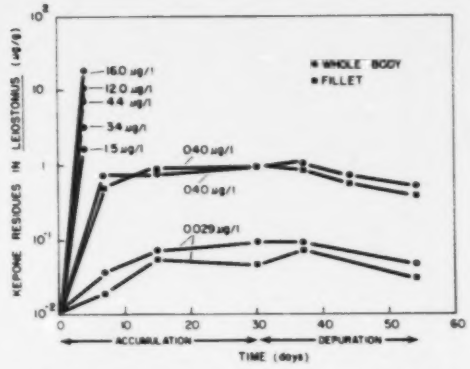

Fig. 5. Bioconcentration of Kepone from water containing average measured concentrations of 0.029, 0.40, 1.5*, 3.4*, 4.4*, 12.0*, and 16.0* µg/l by spot (*Leiostomus xanthurus*) exposed for 4 or 30 days, and its depuration by fish placed in Kepone-free water for 24 days (mean temperature 23°C; mean salinity 18 ‰). *Data from Schimmel and Wilson (1977).

tained an average of twenty mysids (Stickney et al. 1974); mysids comprised up to 14% of the diets of striped bass from the York and Rappahannock rivers. Mysids were conspicuously absent in gut analyses of James River striped bass, but decapod crustaceans (i.e. *Palaemonetes* sp.) accounted for 48% (by volume) of their diets (Markel and Grant 1970). *Palaemonetes* have one of the highest bioconcentration factors of Kepone (Table 2), and like other decapod crustaceans, are one of the species least sensitive to acute exposures of Kepone (Schimmel and Wilson 1977). Grass shrimp bioconcentrated Kepone up to 11,000 times the concentration in the exposure water. After 28 days of exposure to 0.023 µg Kepone/l, each shrimp contained approximately 8.6 ng Kepone, an amount that could be transferred to predators. Bioconcentration of Kepone was more efficient with increased concentrations in water for all crustaceans tested (Table 2).

Kepone was bioconcentrated from water by sheepshead minnows – important omnivores that link energy transfer from detritus and benthic plants and animals to carnivores in higher trophic levels. Each fish (mean weight 1.5 g) contained approximately 0.54 µg Kepone after 28 days of exposure to 0.05 µg Kepone/l seawater. Kepone concentrations were slightly higher in female sheepshead minnows (0.35 µg/g) than male fish (0.25 µg/g).

Spot, a commercially valuable food fish, bioconcentrated Kepone from 0.029 µg/l seawater; each fish (mean weight 1.4 g) contained approximately 0.13 µg Kepone. The bioconcentration factors for Kepone in fish were similar to those of other chlorinated hydrocarbon insecticides (Schimmel et al. 1975; Schimmel et al. 1976). Kepone accumulated in edible fillets to near the whole-body concentrations in fish (Figs. 4, 5,& 6); therefore, one of the largest reserves (22 %) of Kepone in absolute weight is in the edible portion of contaminated fish. Although the greatest body burdens of Kepone on a wet-weight basis are in the brain, liver, and gill tissues, the relatively large size of muscle and offal tissues contributes large Kepone reserves to higher trophic levels (Fig. 6).

Depuration of Kepone was not consistent among the species tested. Clearance of the chemical from oysters was relatively rapid, with no Kepone detected within 7 to 20 days after exposure ceased (Fig. 1). Depuration of Kepone from laboratory exposed grass shrimp (Fig. 3) and fish (Fig. 4 & 5) was slower; Kepone concentrations were reduced 30–50% in 24 to 28 days. Grass

TABLE 1. Concentrations of Kepone (μg/g wet tissue) measured in oysters (*C. virginica*) exposed to 0.03 or 0.39 μg/l for 28 days, mysids (*M. bahia*) exposed to 0.026 or 0.41 μg/l for 21 days, grass shrimp (*P. pugio*) exposed to 0.023 or 0.40 μg/l for 28 days, sheepshead minnows (*C. variegatus*) exposed to 0.05 μg/l for 28 days, and spot (*L. xanthurus*) exposed to 0.029 or 0.4 μg/l for 30 days in flowing water experiments. Animals were allowed to depurate Kepone for up to 28 days in Kepone-free flowing seawater.

Duration of Exposure (Days)	Oysters 0.03 μg/l	Oysters 0.39 μg/l	Mysids 0.026 μg/l	Mysids 0.41 μg/l	Shrimp 0.023 μg/l	Shrimp 0.40 μg/l	Duration of Exposure (Days)	Minnows 0.05 μg/l	Minnows 0.05 μg/l	Spot 0.029 μg/l	Spot 0.029 μg/l	Spot 0.40 μg/l	Spot 0.40 μg/l
	Whole Body	Whole Body	Whole Body	Whole Body	Whole Body	Whole Body		Muscle	Whole Body	Muscle	Whole Body	Muscle	Whole Body
1/6	0.012	0.19					1/6						
1/3	0.031	0.53					1/3						
1	0.036	0.96	–	–	–	–	1	<0.01	<0.01				
2	–	–	–	–	<0.02	0.27	2						
3	–	–	–	–	–	–	3	0.019	0.031				
4	0.11	1.3	<0.04	2.0			4						
7	–	–	0.16	3.3	0.038	0.47	7	0.086	0.14	<0.02	0.037	0.50	0.73
8	0.18	2.2					8						
9	–	–	–	–	0.072	1.79	9						
11	–	–	0.15	6.3			11						
12	0.21	2.4					12						
14	–	–	0.19	4.7	0.088	2.18	14	0.19	0.22				
15	0.20	2.5	–	–	–	–	15	–	–	0.055	0.072	0.86	0.76
19	0.29	3.5					19						
21	0.23	3.6	0.12*	4.8*	0.12	3.15	21	0.21	0.24				
25	0.19	2.7					25						
28	0.21*	2.2*	–	–	0.087*	4.57*	28	0.29*	0.37*				
30	–	–	–	–	–	–	30	–	–	0.048*	0.093*	0.99*	0.94*
Duration of Depuration							**Duration of Depuration**						
1/6	0.30	2.1					1/6						
1/3	0.30	2.7					1/3						
1	0.14	1.4					1						
2	–	–	–	–	0.1	2.62	2						
4	0.074	0.18					4						
7	<0.01	0.18	–	–	0.1	2.5	7	0.21	0.22	0.074	0.093	0.87	1.04
11	<0.01	0.055					11						
14	<0.01	0.039	–	–	0.084	1.78	14	–	–	–	–	0.57	0.72
21	<0.01	0.039	–	–	0.055	1.5	21						
24	–	–	–	–	–	–	24	–	–	0.031	0.0469	0.38	0.52
28	<0.01	<0.01	–	–	0.035	0.84	28						

* Final day of exposure.

TABLE 2. Bioconcentration factors for selected species exposed to measured concentrations of Kepone in water.

Species	Exposure Concentration (μg/l)	Duration of Exposure (days)	Mean Bioconcentration Factor
Chlorococcum sp.	100. (static)	1	340
Chlorococcum sp.	40. (injection)	2	6,000
Crassostrea virginica	0.03	19	9,354
Crassostrea virginica	0.39	21	9,278
Artemia salina	5. (static)	2	10
Artemia salina	100. (static)	2	23
Mysidopsis bahia	0.026	21	5,962
Mysidopsis bahia	0.41	21	13,473
Palaemonetes pugio	0.023	28	5,127
Palaemonetes pugio	0.4	28	11,425
Cyprinodon variegatus	0.05	28	7,115
Leiostomus xanthurus	0.029	30	3,217
Leiostomus xanthurus	0.4	30	2,340
Leiostomus xanthurus	1.5*	4	1,120
Leiostomus xanthurus	3.4*	4	941
Leiostomus xanthurus	4.4*	4	1,591
Leiostomus xanthurus	12.0*	4	900
Leiostomus xanthurus	16.0*	4	1,050

* Data from Schimmel and Wilson (1977).

shrimp from the Lafayette River depurated Kepone at rates similar to those of laboratory exposed shrimp with approximately 20% of the Kepone lost during 21 days in seawater containing no Kepone. Spot that were exposed to Kepone for 30 days and allowed to depurate for 24 days, contained highest Kepone concentrations in brain, followed by liver, gills, and muscle (Fig. 6). Many chlorinated chemicals are highly concentrated in liver and other fatty tissues (Parrish et al. 1974; Parrish et al. 1975), but the unusual distribution of Kepone might be explained by its water solubility relative to other insecticides.

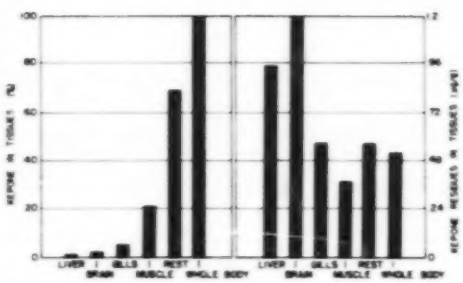

Fig. 6. Distribution of Kepone in selected tissues of spot (*Leiostomus xanthurus*). Spot were exposed to 0.4 μg Kepone/l for 30 days and allowed to depurate and equilibrate for an additional 24 days prior to sampling (mean temperature 23°C; mean salinity 18 ‰). Percentage of whole-body Kepone burden in five tissues is on left. Measured residues (wet weight) are on right.

Bioaccumulation of Kepone in Food Chains Consisting of Estuarine Organisms

Algae-Oyster Food Chain

Chlorococcum, grown in media enriched to 0.1 mg Kepone/l, bioconcentrated the chemical to a mean of 34 μg/g in whole cells; the bioconcentration factor in these static exposures was 340X (Table 2). In preliminary tests, continuous infusion of 2.1 μg Kepone/hour into static cultures of *Chlorococcum* produced cells with bioconcentration factors near 6000 × within 48 hours; adsorption of Kepone by the cells and vessels limited the average measured concentration in the media to approximately 0.04 mg/l. Continuous exposure in this test is probably more representative of those occurring in an estuary, and places the importance of bioconcentration of Kepone by phytoplankton in proper perspective.

Oysters bioaccumulated Kepone to 0.21 μg/g when fed *Chlorococcum* sp. containing an average of 34 μg Kepone/g for 14 days (Fig. 7; Table 3). Kepone in feces and pseudofeces from these oysters averaged 1.78 μg Kepone/g (dry weight). Kepone was not detectable in the unfiltered water samples; shell growth was evident; and all oysters gained weight during the test. Weight gain

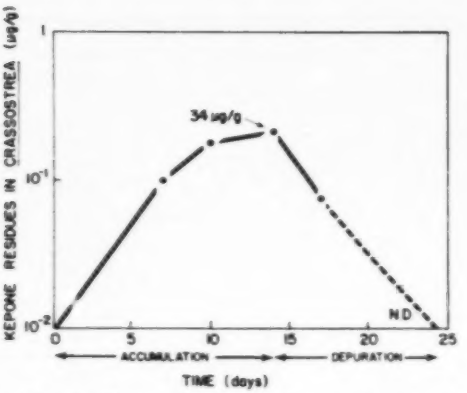

Fig. 7. Bioaccumulation of Kepone by oysters (*Crassostrea virginica*) that consumed algae (*Chlorococcum* sp.) with residues of 34 µg Kepone/g (wet weight). Oysters fed on contaminated algae for 14 days (mean water temperature 22°C; mean salinity 19 ‰), and were then fed uncontaminated plankton for an additional 10 days to allow depuration of the chemical. ND = not detectable, <0.02 µg/g wet weight.

in exposed oysters was not different than control oysters (analysis of covariance, $\alpha = 0.05$). Kepone concentrations in oysters fed the contaminated algae appeared to almost reach equilibrium in 14 days; but quantity of Kepone transferred from these algae to oysters was limited, probably due to rapid depuration of the chemical from the oysters. Kepone was not detectable (<0.02 µg/g) 10 days after the oysters received no contaminated food. Most Kepone was depurated from oysters within 96 hours; therefore, if oysters in the natural environment contain measurable Kepone residues, a recent or continuous source of Kepone from water and/or food has been available.

The maximum overall accumulation and transfer of Kepone or food-chain factor from water to algae and finally to oysters was 2.1 (Table 4). The food-chain factor was obtained by dividing the concentration of the contaminant in the final consumer by the concentration of the contaminant in the water of the primary producer. The concentration measured in each consumer can be compared with a lower trophic level to determine the bioaccumulation factor of the contaminant for that predator-prey pair. Bioaccumulation factor is similar to bioconcentration factor, but the contaminant is in food and is consumed by the predator. The

TABLE 3. Concentrations of Kepone (µg/g whole body, wet tissue) measured in oysters (*C. virginica*) fed algae (*Chlorococcum* sp.) containing 34 µg/g for 14 days and in spot (*L. xanthurus*) fed mysids (*M. bahia*) containing 0.02 µg/g (estimated) or 1.03 µg/g for 30 days in flowing water experiments. Oysters were fed uncontaminated plankton for an additional 10 days to allow depuration of the chemical.

Duration of Exposure (Days)	Oysters fed 34 µg/g	Spot fed 0.02 µg/g (Estimated)	Spot fed 1.03 µg/g
	µg/g Whole Body	µg/g Whole Body	µg/g Whole Body
0	<0.02	<0.02	<0.02
5	–	<0.02	0.095
7	0.10	–	–
10	0.18	<0.02	0.15
14	0.21*	–	–
20	–	<0.02	0.59
30	–	0.015,0.024*	1.0,1.1*
Duration of Depuration			
3	0.075	–	–
10	<0.02	–	–

* Final day of exposure.

TABLE 4. Kepone transfer in algae-oyster food chain. Algae (*Chlorococcum* sp.) grown in Kepone enriched media for 24 hrs was fed to oysters (*C. virginica*) for 14 days in flow-through feeding experiment.

	Control food chain	Exposed food chain
(1) Kepone (single dose) in algal media (mg/l)	Control	0.1
(2) Kepone residues in algae after 24 hrs of exposure (mg/kg)	Control (ND)*	$\bar{X}$ = 34
(3) Bioconcentration factor from water [(2)/(1)]	–	340
(4) Kepone residues in oysters after 14 days of feeding	Control (ND)*	0.21
(5) Bioaccumulation factor from algae to oysters [(4)/(2)]	–	.007
(6) Food chain factor [(4)/(1)]	–	2.1

* ND = non-detectable (<0.02 mg/kg).

bioaccumulation factor for oysters consuming algae under these test conditions was only 0.007 (Table 4). These data indicate that transfer of Kepone from algae by oysters was inefficient, or that the uptake was masked by the oyster's ability to depurate the chemical quickly.

Plankton-Mysid-Fish Food Chain

Spot accumulated Kepone by consuming live mysids that had grazed on Kepone-laden brine shrimp (Fig. 8; Tables 3, 5). Brine shrimp exposed to 0.1 mg Kepone/l seawater contained whole-body residues of 2.33 μg/g after 48 hours. Mysids that fed for 72 hours on these brine shrimp contained 1.23 μg Kepone/g. Kepone concentrations in spot that consumed the mysids for 30 days were slightly less than those in the mysids (Fig. 9), but uptake of Kepone exceeded depuration in fish as indicated by the positive slope of the uptake curves. The failure for residues to reach equilibrium during the test could be attributed to the slow depuration of Kepone from fish tissues.

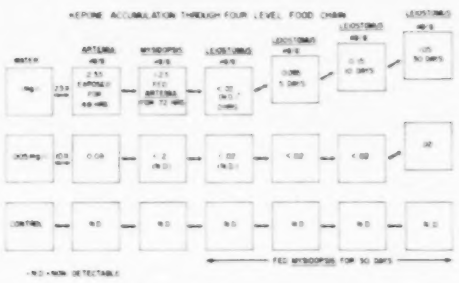

Fig. 8. Block diagram of four-level food chain. Nominal concentrations of Kepone in water (left) were control (bottom), 0.005 mg/l (center), and .1 mg/l (top). *Artemia salina* nauplii, hatched in these concentrations of Kepone, were fed to mysids (*Mysidopsis bahia*), and mysids were fed to spot (*Leiostomus xanthurus*) for 30 days. Average measured concentrations of Kepone in each trophic species is given. Mean water temperature was 19°C and mean salinity 18 ‰.

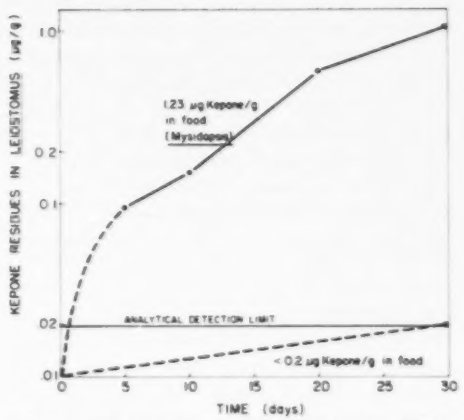

Fig. 9. Bioaccumulation of Kepone by spot (*Leiostomus xanthurus*) fed contaminated mysids (*Mysidopsis bahia*) containing average measured whole-body residues of <.2 (0.023 estimated) or 1.23 μg Kepone/g wet tissue. Detection limit for Kepone in spot tissues was ≥0.02 μg/g. Mean water temperature was 19°C and mean salinity 18 ‰.

TABLE 5. Kepone transfer in plankton-mysid-fish food chain. Brine shrimp (*A. salina*) were hatched during 48 hrs in Kepone enriched seawater and were fed to mysids (*M. bahia*) for 72 hrs. Mysids were then fed to spot (*L. xanthurus*) for 30 days in flow-through feeding experiment.

	Control food chain	Low Exposure food chain	High Exposure food chain
(1) Kepone (single dose) in brine shrimp media (mg/l)	Control	0.005	0.1
(2) Kepone residues in brine shrimp after 48 hrs of exposure (mg/kg)	Control (ND)*	0.049 0.043 0.058 $\bar{x}$ = 0.050	1.3 2.4 3.3 $\bar{x}$ = 2.33
(3) Bioconcentration factor from water [(2)/(1)]	–	10.	23.3
(4) Kepone residues in mysids after 72 hrs of feeding (mg/kg)	Control (ND)*	$\bar{x}$ = 0.023 (estimated)	0.89 1.0 1.8 $\bar{x}$ = 1.23
(5) Bioaccumulation factor from brine shrimp to mysids [(4)/(2)]	–	0.5 (estimated)	0.53
(6) Kepone residues in spot after 30 days of feeding (mg/kg)	Control (ND)*	0.015 0.024 $\bar{x}$ = 0.0195	1.0 1.1 $\bar{x}$ = 1.05
(7) Bioaccumulation factor from mysids to spot [(6)/(4)]	–	>0.85 (estimated)	>0.85
(8) Food chain factor [(6)/(1)]	–	>3.9	>10.5

* ND = non-detectable (<0.02 mg/kg).

Mysids, which consumed *Artemia* with residues of 0.05 or 2.33 μg Kepone/g (wet weight), attained 0.023 (estimated) or 1.23 μg Kepone/g whole-body residues within 72 hours. The estimated 0.023 μg Kepone/g whole-body mysids, obtained by assuming the bioaccumulation factor of 0.85 (Table 5) for Kepone transfer from mysids to fish as observed in the food chain beginning with 0.1 mg/l, also occurred in the food chain that began with 0.005 mg Kepone/l. The residue in mysids was then estimated to be 0.023 μg Kepone/g, which was consistent with the estimated bioaccumulation factor of 0.5 for Kepone transfer from *Artemia* to mysids for 72 hours. The food-chain factors were different for this food chain (3.9 compared to 10.5), since the bioconcentration factor for Kepone by brine shrimp from the lower concentration in water (0.005 mg/l) was less than that from the higher concentration (0.1 mg/l). The initial bioconcentration of Kepone from water by planktonic food organisms was the dominant source of Kepone to each member of this food chain, since bioaccumulation factors were less than unity.

In the field, lower concentrations of Kepone in seawater could result in a duplication of Kepone residues found in the fish of this food chain because the plankton could be expected to be chronically exposed to the contaminant. Thus, the food-chain factor would be expected to increase in the natural environment since bioconcentration factors for a chlorinated hydrocarbon pesticide (DDT) in feral plankton have been shown to exceed 4000X (Cox 1971). However, bioconcentration of Kepone could overshadow the amount of the chemical received from food by the animals in this food chain. Approximately 3,000 times as much Kepone in food as in water was needed to produce similar concentrations in spot in 28 to 30 days. Therefore, bioconcentration of Kepone was dominant in this food chain, but significant quantities (>85%) of Kepone transferred from prey to predatory fish. Rapid uptake from water and food, slow depuration, and appreciable solubility in water indicate that Kepone will transfer through food webs and pose threats to consumers.

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